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I hereby certify that on the date specified below, this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to the Commissioner for Patents, Washington, DC 20231.

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Jennifer L. Mitcham et al.
Application No.	:	09/636,801
Filed	:	August 10, 2000
For	:	COMPOSITIONS AND METHODS FOR THERAPY AND DIAGNOSIS OF OVARIAN CANCER
Examiner		M. Zeman
Art Unit		1631
Docket No.		210121.462C4
Date		December 11, 2001

DECLARATION OF GARY R. FANGER, PH.D.

Assistant Commissioner of Patents
Washington D.C. 20231

The undersigned, Gary R. Fanger, Ph.D., hereby declares:

1. I am a Scientist and Project Group Leader at Corixa Corporation, the assignee of the subject application. The following experiments were performed under my supervision.

2. IMMUNOHISTOCHEMICAL EVALUATION OF O8E:

To evaluate the tissue specificity of O8E, monoclonal antibodies (mAbs) were generated against recombinant O8E (rO8E) protein, the sequence of which corresponds to SEQ ID NO:392. To generate these antibodies, either normal Balbc mice or Medarex mice were i.p. immunized repeatedly with rO8E protein. Spleens from mice with positive titers to O8E were then collected and used for fusion to myeloma cells in order to generate B cell hybrids. Supernatants from the resulting hybrids were tested by ELISA for specificity to rO8E. For ELISA analysis, 96 well plates were coated with

rO8E at a concentration of 1-2μg/ml or 10μg/ml then allowed to incubate for 60 minutes at room temperature. After coating, the plates were washed five times with PBS containing 0.1% Tween-20 and then blocked with PBS containing 0.5% BSA and 0.4% Tween-20 for 1-2 hours at room temperature. Following the addition of hybridoma supernatants or purified mAb, the plates were incubated for an additional 60 minutes at room temperature. The plates were then washed as above and mouse anti-human IgG-HRP linked secondary antibody (Pharminigen) was added and incubated for 60 minutes at room temperature, followed by a final washing step as above. TMB peroxidase substrate was then added to the plates and incubated for 5-15 minutes at room temperature in the dark. The reaction was stopped by the addition of 1N H_2SO_4 and the OD was read at 450nm.

Antibodies that were shown to react specifically against the rO8E protein were then used to evaluate the tissue specificity of O8E using immunohistochemical (IHC) analysis. To perform IHC analysis, paraffin-embedded formalin fixed tissues from both ovarian tumors (n=5) and normal ovary (n=5) were sliced into 8 micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with PBS containing 10% serum for 5 minutes. The O8E mAbs were then added to each section for 25 minutes, followed by a second 25 minute incubation with an anti-mouse biotinylated antibody. Endogenous peroxidase activity was blocked by three 1.5 minute incubations with hydrogen peroxidase. The avidin biotin complex/horse radish peroxidase system was used along with DAB chromogen to visualize antigen expression. Slides were counterstained with hematoxylin. Results demonstrated that, O8E expression was detected in 60% of ovarian tumors tested but was not detected in any of the normal ovarian tissue samples tested.

The expression profile of O8E, *i.e.*, its over expression in ovarian tumors relative to normal ovarian tissue, demonstrates that it is an ovarian cancer marker which would be useful in the detection and diagnosis of ovarian cancer.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are

believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Gary R. Fanger, Ph.D. Date 12/16/01

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